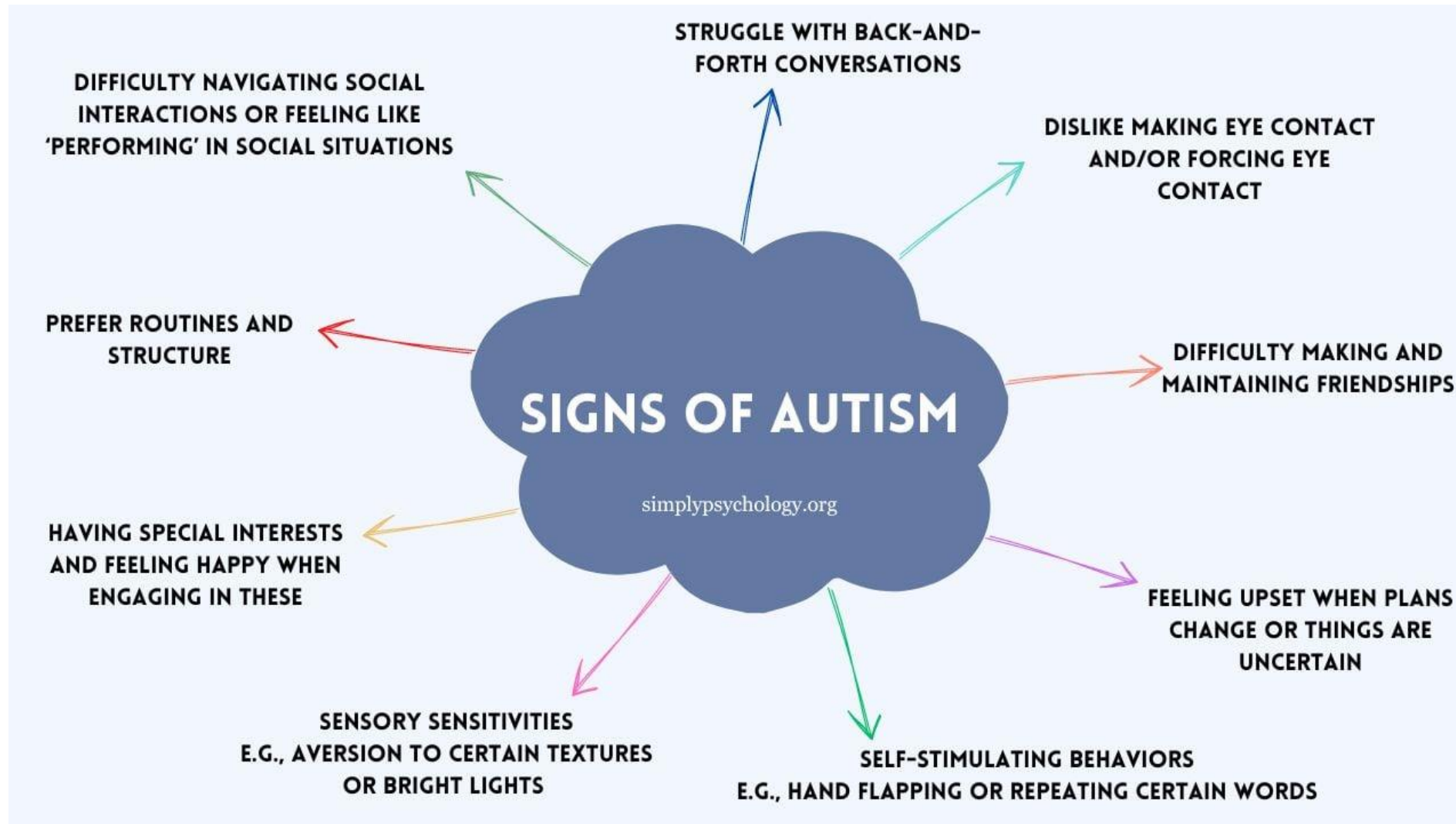


**Multi-omics data integration and machine learning (ML) explaining the molecular basis of the complex microbiota-gut-brain axis and predicting possible biomarkers of autism**

Alessandra Mezzelani  
National Research Council-Institute for Biomedical Technologies (CNR-ITB),  
Italy

EPTRI Scientific Meeting– Bari – 18-07-2024

# Autism Spectrum Disorders (ASD)



# Epidemiology

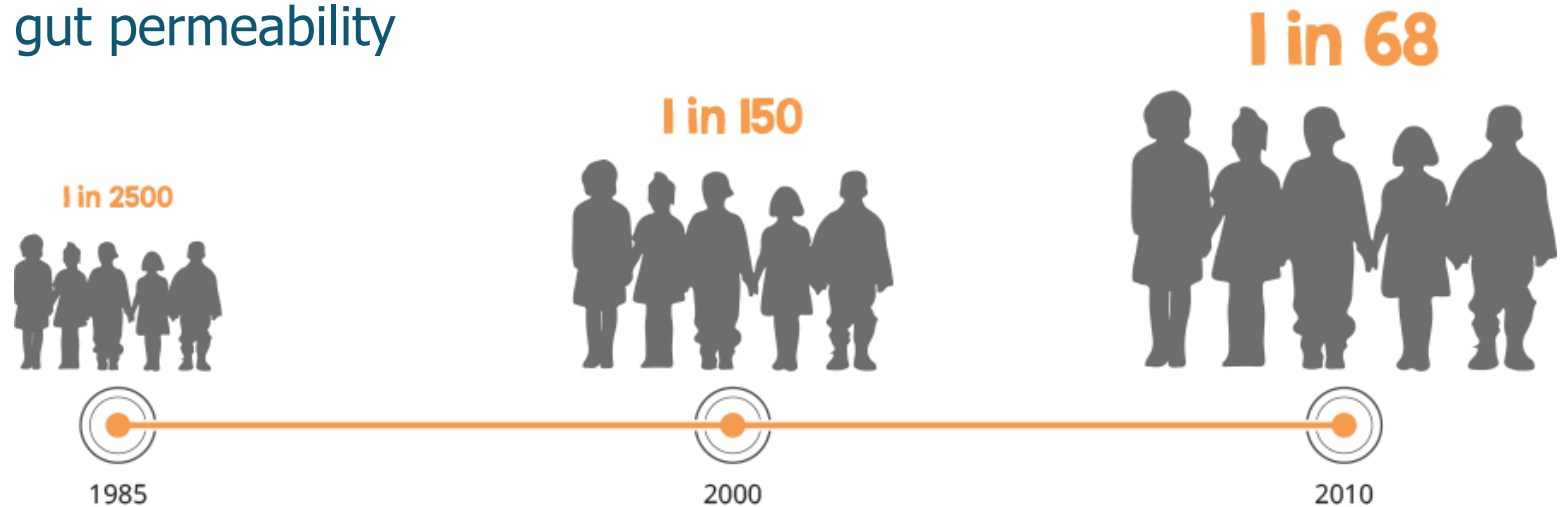
manifests by 3-5 years of age (also regressive)

male:female=5:1

only 30% of cases display a causative genetic variations

co-occurring medical conditions

- syndromes
- mental retardation
- inflammation
- epilepsy
- GI disorders, dysbiosis, gut permeability



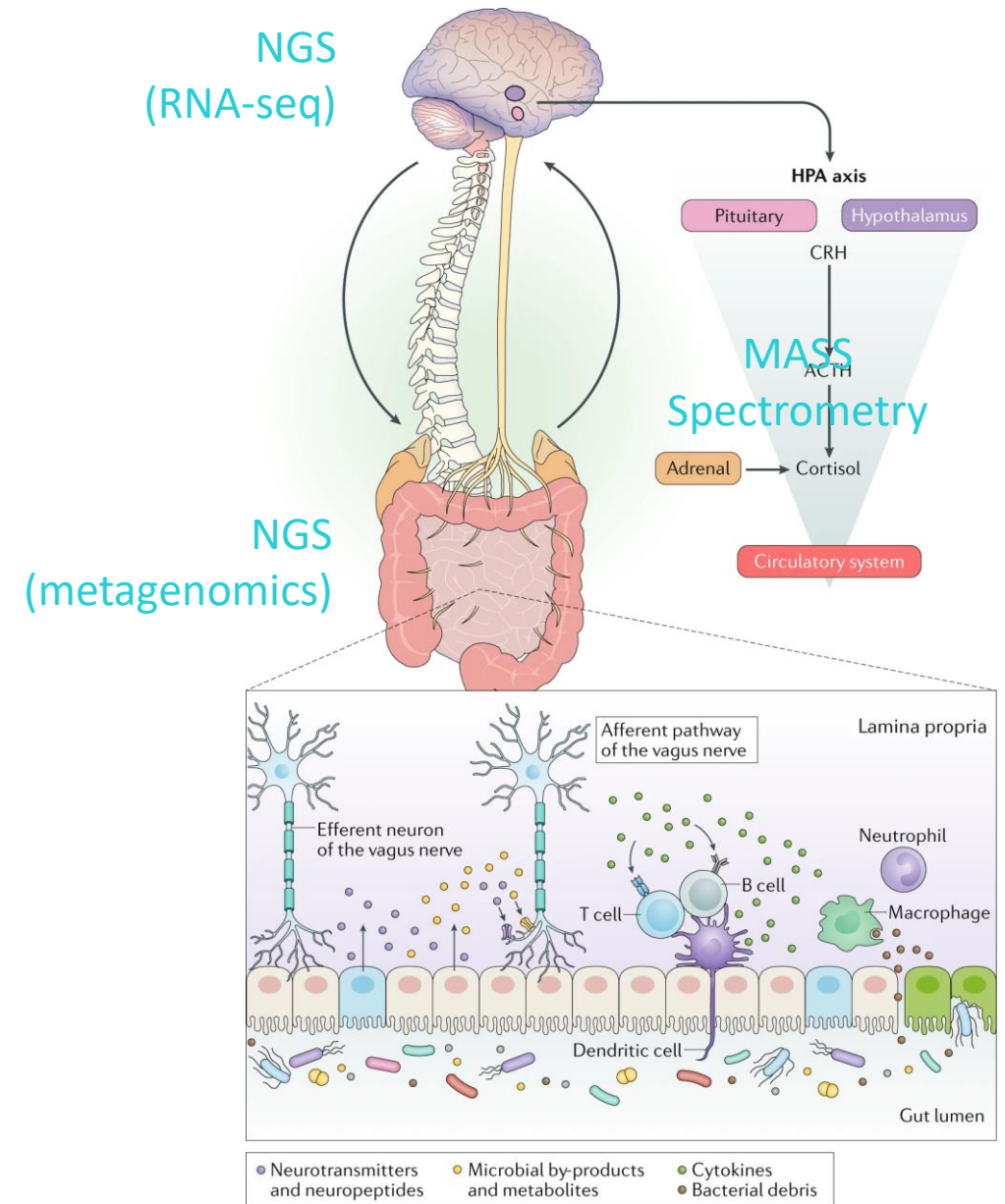
# The study of the microbiome-gut-brain axis needs a multi-level approach

Gut-Brain Axis (GBA):  
interplay between the gut and processes controlled by neuroendocrine, neuroimmune and autonomous nervous systems.

**GBA impairment is implicated in ASD**  
Systemic process that **demands a multi-omics approach**

## Challenges in multi-omics approaches

- multiple samples for each subject
- data collection by various technologies (NGS, MS)
- interaction of issues among omics



# A recent work used pathways to connect evidence from brain and gut

nature neuroscience

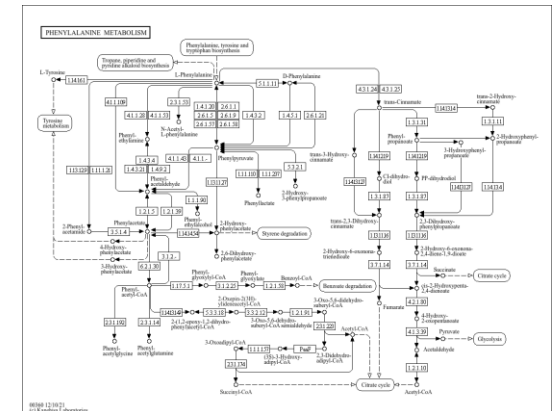
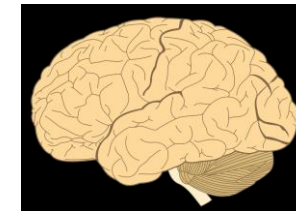


Article

<https://doi.org/10.1038/s41593-023-01361-0>

## Multi-level analysis of the gut–brain axis shows autism spectrum disorder-associated molecular and microbial profiles

Organism	Body type	Data type	Number of studies	Number of subject pairs
Human	Postmortem brain tissue	RNAseq	4	49
Human	Serum	Immune markers	1	22
Human	Serum	Metabolome	2	50
Human	Urine	Metabolome	1	26
Human	NA	Dietary survey	1	26
Human	NA	Behavioral survey	1	28
Microbial	Fecal	Metabolome	2	43
Microbial	Fecal	16S amplicon	10	346
Microbial	Fecal	Shotgun metagenomics	3	83



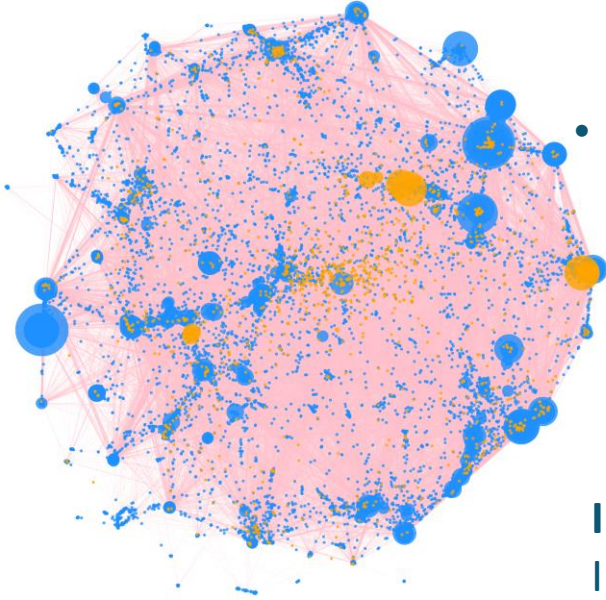


# Can we use biological networks to map evidence from brain and gut?



## Data: ASD vs neurotypical subjects

- Predisposition (SFARI)
- Brain gene activity (RNAseq from post-mortem brain tissues, 4 cohorts)
- Gut microbiota (Shotgun metagenomics from faeces, 3 cohorts)



## Interactome

Interactions among human genes and between human and gut microbial genes:

18'785 genes, 183'654 interactions

Data sources: STRING, HBNNet

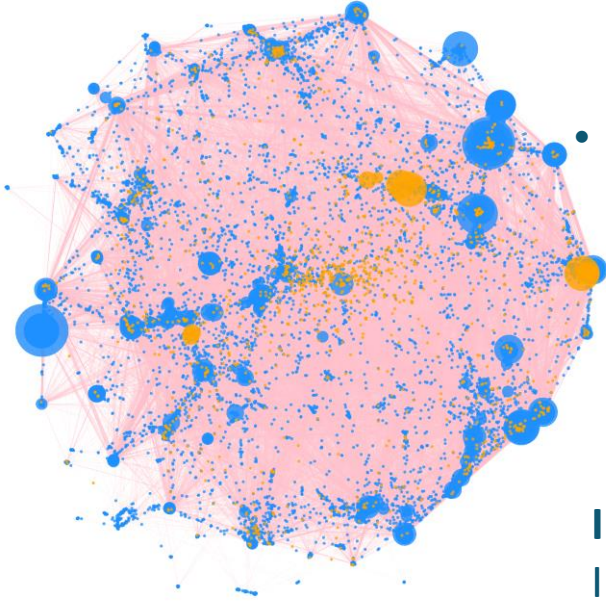


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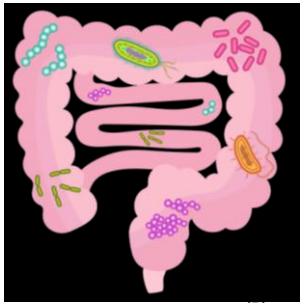


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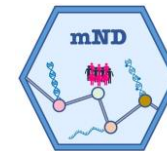
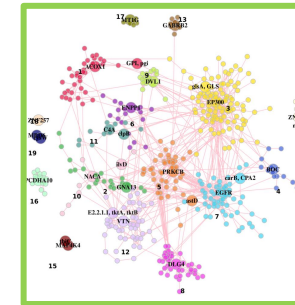
Data sources: STRING, HBNNet



## APPROACH

We used network analysis tools to extract gene networks that connect altered genes to one another

<https://github.com/emosca-cnr>

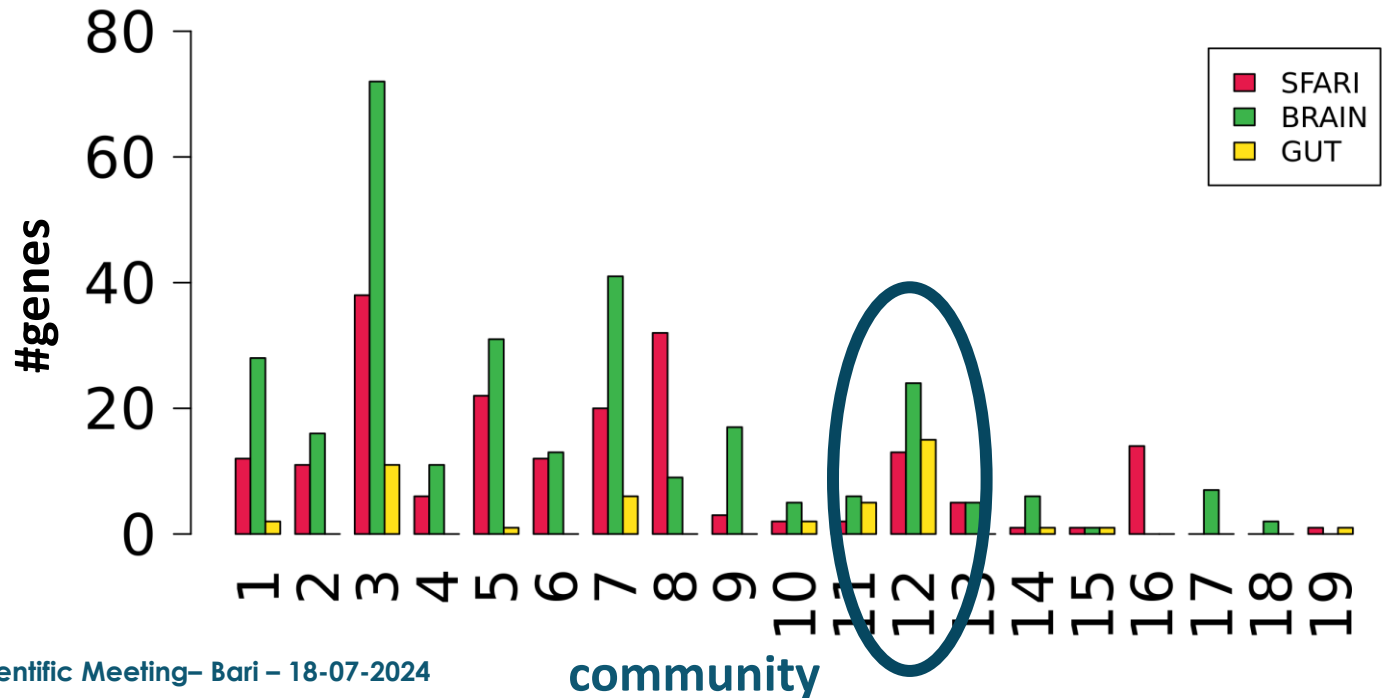
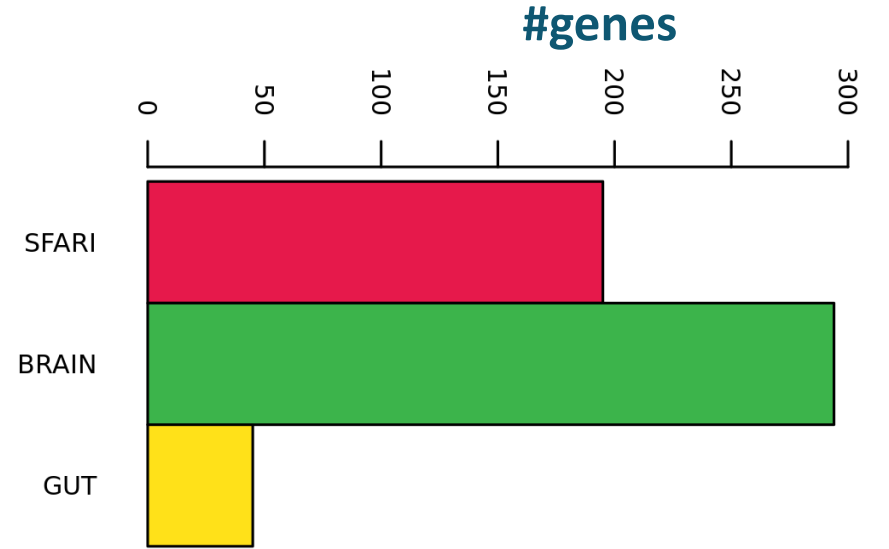
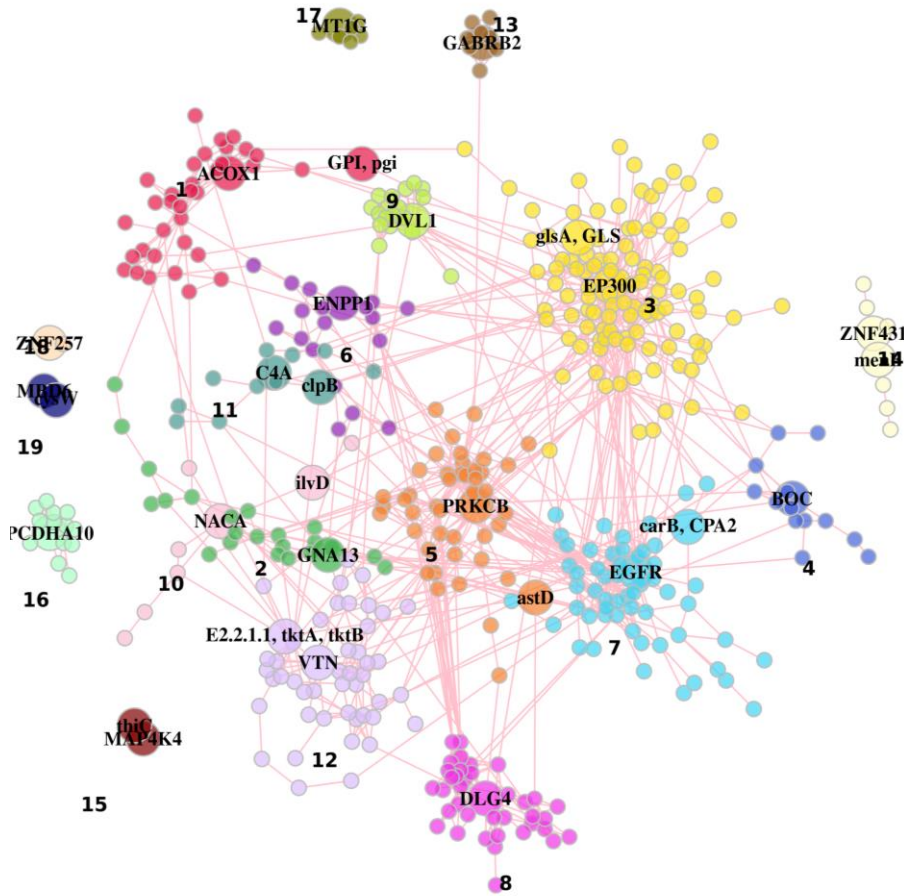


# The top network is modular and connects the three types of evidence

Network of 472 genes and 1470 links

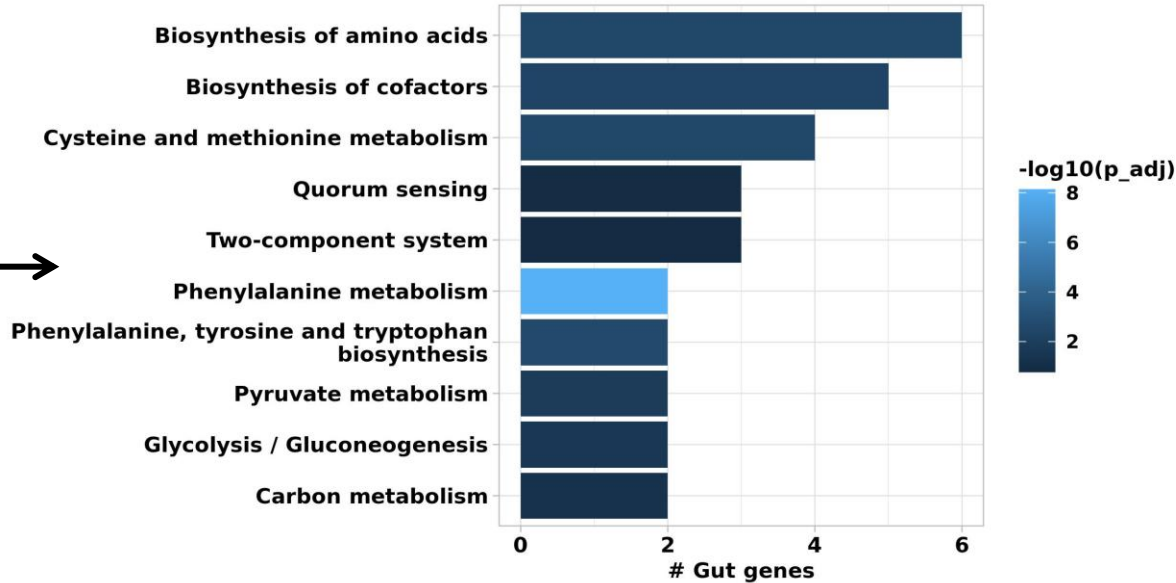
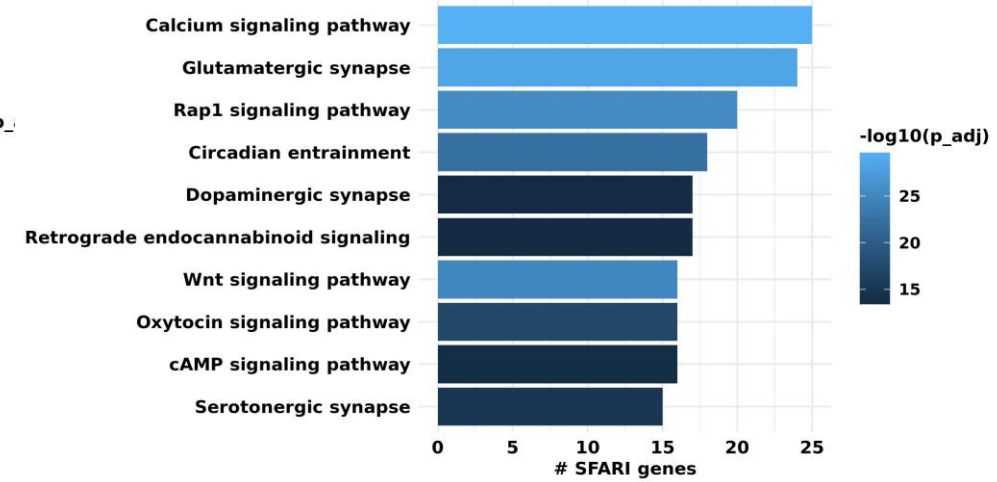
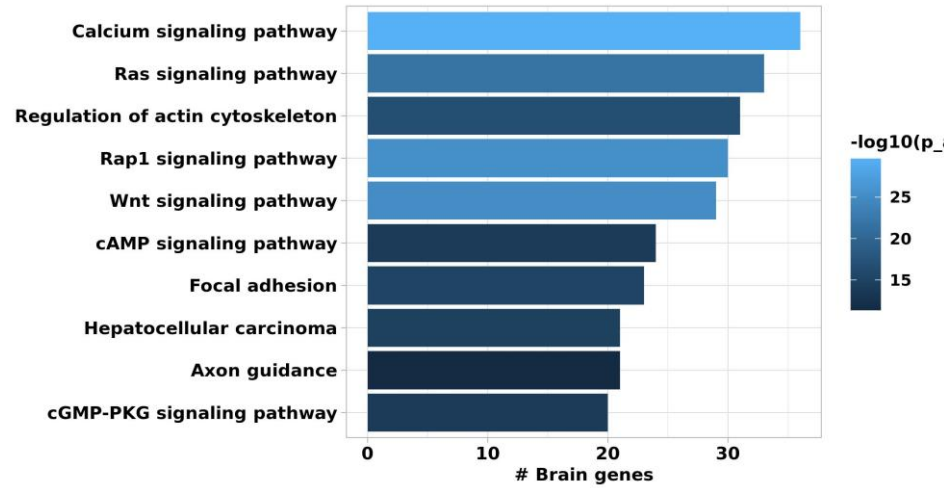
It has a modular structure ( $Q = 0.74$ )

suggesting the involvement of various pathways/functions





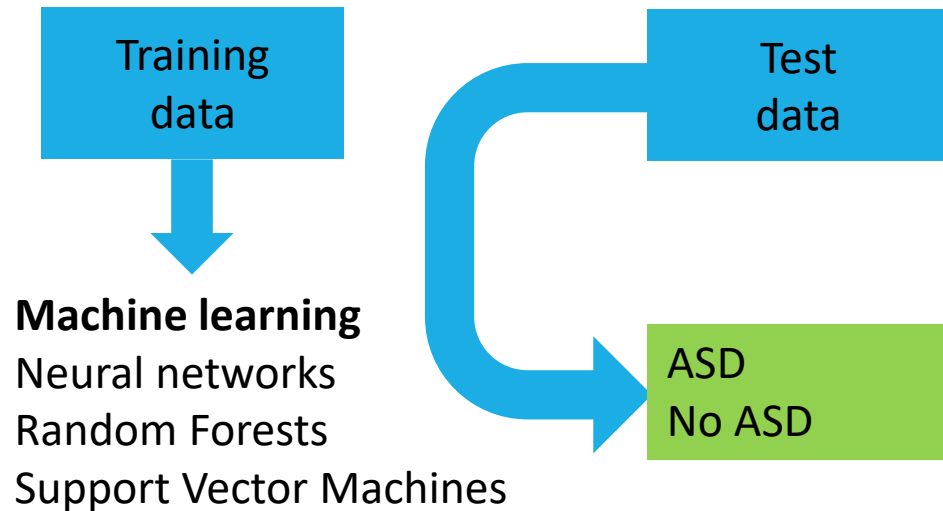
# Pathways enriched in top network genes



1. Many genes supported by both SFARI and brain RNA-seq
2. Pathways enriched in microbial genes control the **metabolism of amino acids** involved in biosynthesis of neurotransmitters (as in Morton et al.)

# ASD prediction and key predictors

Are the identified genes able to distinguish ASD and neurotypical subjects?



We obtained AUC ranging from 0.5 to 0.8. This variation can be brought back to various sources of heterogeneity (e.g., samples size, batch effects, technological platforms).

Dataset	AUC (Train)	AUC (Test)
SRP132816	0.88	0.68
All RNAseq datasets	0.81	0.73
Wang2020	0.81	0.82
Dan2020	0.82	0.82
Averina2020	0.57	0.51
All SMS datasets	0.7	0.55

Most important features for prediction come from communities



# Thanks

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Matteo Gnocchi

Alessandra Mezzelani, PhD

